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“Mätningar av PFOS i bröstmjölk och blod”

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Anna Kärrman, Ingrid Ericson, Bert van Bavel och Gunilla Lindström.

MTM Forskningscentrum
Örebro Universitet

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Projektledare Gunilla Lindström
Analysis and Occurrence of Perfluorinated Chemicals in Breast Milk and Serum from Swedish Women, 1996-2005

Anna Kärrman*, Ingrid Ericson, Bert van Bavel, Gunilla Lindström.

Man-Technology-Environment (MTM) Research Centre, Örebro University, SE-701 82 Örebro, Sweden

*Telephone: +46 19 30 14 01. Fax: +46 19 303566. Email:anna.karrman@nat.oru.se

Abstract

Breast milk samples and blood serum collected from primipara women in Sweden during the period 1996 to 2004 were analyzed with the aim to study levels of perfluorinated chemicals (PFCs) and the concentration ratio between milk and blood.

A total of five perfluorinated chemicals (PFCs) were detected in breast milk, of which perfluorooctanesulfonate (PFOS) and perfluorohexanesulfonate (PFHxS) were found most frequently. In addition, perfluorooctanesulfonamide (PFOSA), perfluorononanoic acid (PFNA) and perfluorooctanoic acid (PFOA) were detected. The highest mean concentration in individual milk samples was obtained for PFOS (0.172 ng/mL) followed by PFHxS (0.068 ng/mL) and PFOSA (0.012 ng/mL). The quantification of PFOA was hampered in most of the samples due to a high procedural blank contamination. A total of eight PFCs were detected in the serum samples.

Breast milk levels in this study were on average 113 times lower compared to serum levels for PFOS, 57 times lower for PFHxS and 23 times lower for PFOSA. There was a strong association between increasing serum concentration and increasing milk concentration for PFOS and PFHxS.

During the period 1996-2004, the levels in pooled breast milk samples were relatively constant with a slightly decreasing trend for the pools collected in years 2003-2004.

Analysis of breast milk, consecutive sampled over a period of several days, did not indicate a decrease in levels with progression of lactation.
1. Introduction

Perfluorinated chemicals (PFCs) have been found in environmental and human samples worldwide as a consequence of over 50 years of production. Their applications and use are as surfactants, as paper, leather and textile protection and as polymers and plastics to mention some. They pose a threat to biota and humans due to their persistence, bioaccumulation and toxicity.

An increasing number of studies show that humans, including Swedish residents, are exposed to a large number of PFCs. Human exposure pathways are currently being investigated. Studies from USA and Australia indicate that younger people have equal or higher PFC serum levels than adults and elder people. The extent of perinatal exposure to PFCs in the general population is not clear today.

A cross-fostering study on Sprague-Dawley rats indicated that perfluorooctane sulfonate (PFOS) can be transferred to pups through breast milk. A study on two breast milk samples from USA showed the presence of PFCs in human milk. PFOS was not detected (detection limit 0.3 ng/mL) but one sample contained 1.6 ng/mL perfluorohexanoic acid (PFHpA) and the other sample 0.8 ng/mL perfluorohexanoic acid (PFHxA). Recently it was reported that low levels (10-592.6 pg/mL) of PFOS, perfluorooctanoic acid (PFOA), PFHxS and perfluorononanoic acid (PFNA) were found in breast milk from women living in China.

PFOS and PFOA are suggested to bind to serum albumin and not to lipids. The lactational transfer mechanism of PFCs and PFC quantity of body burden or blood levels transferred to breast milk is not clear. This study reports occurrence and levels of PFCs in breast milk from Swedish primipara women together with their current blood serum levels at the time of donation. In addition, a temporal trend study with pooled breast milk samples collected from 1996 to 2004 was conducted.

2. Materials and Methods

2.1 Samples. Individual breast milk and serum samples from 12 women were collected in Uppsala, Sweden in the year 2004. Nine composite breast milk samples, collected from three regions (Lund, Göteborg, Lycksele) in year 1996-2004, together with five individual breast milk samples from Örebro, Sweden, sampled in 2003-2005, were also included in the study. Three mothers from Örebro donated consecutive samples. All samples were from primipara women. Samples from Uppsala, Lund, Göteborg and Lycksele were collected during the third week after delivery and stored at –20°C in glass bottles. Samples from Örebro were collected at different occasions, between 0-6 months after delivery and stored at –20°C in different plastic containers.

2.2 Chemicals. Ammonium acetate (>99%, pa for HPLC) was purchased from Fluka (Steinheim, Germany), formic acid (98-100%) from Scharlau (Barcelona, Spain), and methanol (HPLC) from Labscan (Dublin, Ireland). All water used was laboratory produced ultra pure water. Ammonium hydroxide (25 % in water) and sodium acetate was purchased from Merck (Darmstadt, Germany). Perfluorobutanesulfonate (PFBuS) tetrabutylammonium...
salt (≥ 98%), PFOS potassium salt (≥ 98%), perfluorodecanoic acid (PFDA; > 97%), and perfluorohexanoic acid (PFHxA; ≥ 97%) were purchased from Fluka. Perfluoroheptanoic acid (PFHpA; 99%), perfluorononanoic acid (PFNA; 97%), perfluorooctanoic acid (PFOA, 96%), perfluorodecanesulfonate (PFDS) ammonium salt (25 wt% in 2-butoxyethanol (37%) in water), perfluoroundecanoic acid (PFUnDA; 95%), and perfluorotetradecanoic acid (PFTDA, 97%) were purchased from Aldrich (Steinheim, Germany and Milwaukee, WI). Perfluorooctanesulfonamide (PFOSA; 97%) and 7H-PFHpA (98%) were purchased from ABCR (Karlsruhe, Germany). 1H,1H,2H,2H-PFOS (THPFOS, purity unknown), and perfluorohexanesulfonate (PFHxS; 98%) were purchased from Interchim (Montlucon, France). 13C4-labeled PFOA, 13C4-labeled PFOS and 13C5-labeled PFNA were from Wellington Laboratories (Guelph, Ontario, Canada).

2.3 Sample preparation. The serum and milk samples were extracted using solid-phase extraction (SPE) and analyzed by liquid chromatography (LC) coupled to a single quadrupole mass spectrometer (MS). 1 mL milk and 0.5 mL serum, respectively were used in the extraction procedure and placed in a polypropylene tube. Internal standards 13C4-PFOA and 13C4-PFOS were added. The samples were Vortex mixed before 2 mL formic acid/water (1:1) was added. The solution was then sonicated for 15 min and centrifuged at 10 000 x g for 30 minutes. The supernatant was extracted on a Waters Oasis® WAX (weak anion exchange) SPE column (200 mg/2 mL), previously conditioned with 2 mL methanol and 2 mL water. The column was washed with 2 mL sodium acetate buffer solution, pH 4, and 2 mL 40% methanol in water. Sodium acetate buffer was not used for the serum samples. SPE cartridges were vented with air under vacuum suction until visual dryness. Perfluorinated compounds were eluted with 1 mL 2% ammonium hydroxide in methanol. After evaporation under a gentle stream of nitrogen the extracts were filtrated through a 0.2 µm polypropylene filter. The final volume for the serum extracts was 500 µl. Milk extracts were further evaporated to 30 µl and 20 µl 2 mM ammonium acetate in water was added. Finally, filtration through a Microcon YM-3 centrifugal filter (Millipore, Billerica, MA, USA) was conducted at 14000 x g for 30 min. Performance standards, 13C5-PFNA and 7H-PFHpA, were added to both milk and serum extracts immediately before injection.

2.4 HPLC-MS analysis. The SPE extracted samples were analyzed using an Agilent 1100 HPLC system (Agilent, Waldbronn, Germany) coupled to an HP 1100 mass spectrometric detector (MSD, Waldbronn, Germany) with an atmospheric electrospray interface operating in negative ion mode. Separation was performed on a Discovery HS C18 (50 x 2.1 mm, 3 µm) column with a guard column of the same material (Supelco, Bellefonte PA, USA). Both columns were kept at 40 ºC. An extra guard column (HyperCarb 4 x 10 mm, 5µm) was inserted between the pump and injector to remove any fluorochemicals originating from the HPLC system. Injection volume was 10 µl and the flow rate was set to 300 µl/min. The mobile phases consisted of 2 mM ammonium acetate in methanol and 2 mM ammonium acetate in water. The HPLC gradient program used started at 35% methanol followed by a 20 min ramp to 90% methanol, a ten minute hold, with a subsequent ten minute washing sequence with 100% methanol, and then reverting to initial conditions allowing seven minutes stabilization time. MS settings used were: nitrogen nebulizer gas temperature 350 ºC, nebulizer gas pressure 20 psi, nitrogen drying gas flow 13 mL/min, capillary voltage 3500 V. Selected ion monitoring measured the [M-H]⁻ ion for the sulfonates and [M-COOH]⁻ ion for the carboxylates at fragmentation voltages given in Table 1.
TABLE 1. Target PFCs and the method performance together with quantification ion and fragmentation voltage settings used in the selected ion monitoring method.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Abbreviation</th>
<th>Molecular formula</th>
<th>Quantification ion (m/z)</th>
<th>Fragmentation voltage (V)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Perfluorobutane sulfonate</td>
<td>PFBuS</td>
<td>C₄F₉SO₃⁻</td>
<td>299</td>
<td>95</td>
</tr>
<tr>
<td>Perfluorohexane sulfonate</td>
<td>PFHxS</td>
<td>C₆F₁₃SO₃⁻</td>
<td>399</td>
<td>130</td>
</tr>
<tr>
<td>Perfluoroctane sulfonate</td>
<td>PFOS</td>
<td>C₈F₁₇SO₃⁻</td>
<td>499</td>
<td>75</td>
</tr>
<tr>
<td>1H,1H,2H,2H-perfluorooctanesulfonic acid</td>
<td>THPFOS</td>
<td>C₁₃H₂₀SO₃H</td>
<td>427</td>
<td>110</td>
</tr>
<tr>
<td>Perfluorodecane sulfonate</td>
<td>PFDS</td>
<td>C₁₀F₂₁SO₃⁻</td>
<td>599</td>
<td>150</td>
</tr>
<tr>
<td>Perfluorohexanoic acid</td>
<td>PFHxA</td>
<td>C₅F₁₁CO₂H</td>
<td>269</td>
<td>65</td>
</tr>
<tr>
<td>Perfluorooctanoic acid</td>
<td>PFHxA</td>
<td>C₅F₁₁CO₂H</td>
<td>319</td>
<td>70</td>
</tr>
<tr>
<td>Perfluorooctanoic acid</td>
<td>PFHxA</td>
<td>C₅F₁₁CO₂H</td>
<td>369</td>
<td>75</td>
</tr>
<tr>
<td>Perfluorononanoic acid</td>
<td>PFNA</td>
<td>C₈F₁₅CO₂H</td>
<td>419</td>
<td>150</td>
</tr>
<tr>
<td>Perfluorodecanoi hydrocarbon</td>
<td>PFDA</td>
<td>C₁₅F₃₀CO₂H</td>
<td>469</td>
<td>83</td>
</tr>
<tr>
<td>Perfluoroundecanoi hydrocarbon</td>
<td>PFUnDA</td>
<td>C₁₅F₃₀CO₂H</td>
<td>519</td>
<td>85</td>
</tr>
<tr>
<td>Perfluorododecanoi hydrocarbon</td>
<td>PFDoDA</td>
<td>C₁₅F₃₀CO₂H</td>
<td>569</td>
<td>90</td>
</tr>
<tr>
<td>Perfluorotetradecanoi hydrocarbon</td>
<td>PFTDA</td>
<td>C₁₅F₃₀CO₂H</td>
<td>669</td>
<td>93</td>
</tr>
<tr>
<td>Perfluorooctanesulfonamide</td>
<td>PFOSA</td>
<td>C₁₃F₃₀SO₃NH₂⁺</td>
<td>498</td>
<td>113</td>
</tr>
<tr>
<td>Perfluoro-1-[1,2,3,4-¹³C₄]octane sulfonate</td>
<td>¹³C₄PFOS</td>
<td>¹³C₄C₁₇F₁₇SO₃⁻</td>
<td>503</td>
<td>75</td>
</tr>
<tr>
<td>Perfluoro-1-[1,2,3,4-¹³C₄]octane acid</td>
<td>¹³C₄PFOA</td>
<td>¹³C₄C₁₇F₁₇CO₂H</td>
<td>372</td>
<td>75</td>
</tr>
<tr>
<td>Perfluoro-1-[1,2,3,4,5-¹³C₅]monanoic acid</td>
<td>¹³C₅PFNA</td>
<td>¹³C₅C₁₇F₁₇CO₂H</td>
<td>423</td>
<td>150</td>
</tr>
<tr>
<td>7H-perfluorooctanoic acid</td>
<td>7H-PFHpA</td>
<td>HC₁₈F₁₇CO₂H</td>
<td>281</td>
<td>75</td>
</tr>
</tbody>
</table>

*Internal standard, i.e. added before extraction. **Performance standard, i.e. added immediately before injection.

2.5 Quantification and Quality Assurance

Quantification was performed using the internal standard method with non-extracted standards dissolved in 35% methanol in water. Minimum five-point calibration curves ranging from 0.2-0.5 to 24-80 ng/mL were used. A one-point calibration standard was produced with every batch of samples and was used to quantify the samples after passed control (within ±15% of theoretical concentration except for PFOSA, which was within ±19%) against the calibration curve. ¹³C₄-PFOS was used as internal standard for the sulfonates and PFOSA. ¹³C₄-PFOA was used for the carboxylates. ¹³C₅-PFNA and 7H-PFHpA were used to monitor the recovery of the internal standards. The recovery was on average 67% and within 50-130% for 78% of all milk sample determinations and within 84-97% for all serum samples.

Recovery, reproducibility and detection limits can be seen in Table 2. Detection limits were defined as the concentration needed to produce a signal three times higher than the noise. Possible contamination was evaluated by extracting a procedure blank (ultra pure water) with every 12 samples and by repeated methanol injections throughout the sample sequence. No contamination from the system was detected. However procedure blank trace levels were
detected for PFOA, PFOS and PFNA. In the case of blank levels (see Table 2), mean blank signal plus three standard deviations of multiple blank injections were subtracted from the calculated concentrations in the samples. A blank corrected concentration was reported provided that the blank level was equal or less than 50% of the uncorrected concentration.

In order to verify the selectivity of the method, all breast milk samples were extracted in duplicates. The second extract volume was kept at 500 µl and injected on a column-switching LC system connected to a triple quadrupole MS system (Micromass QuattroII, Altrincham, UK). A total of 200 µL was injected on a C18 guard column with a flow rate of 300 µL and using water/methanol mobile phases containing 10 mM ammonium acetate. Gradient separation was achieved using the same column as previously described. Multiple reaction monitoring (MRM) was conducted measuring [M-H] > 80 for the sulfonates and [M-H] > [M-COOH]⁻ for the carboxylates.

Successful participation was achieved in the 1st interlaboratory study on PFCs 17.

### TABLE 2. Recovery and repeatability performance of the method for extracting serum and breast milk samples together with the method detection limits (not including blank levels) and the blank levels (mean + 3 standard deviations) detected during sample analysis, injecting 10 µl of a procedural blank.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Recovery % (CV%)</th>
<th>Detection limit (ng/mL)</th>
<th>Blank concentration (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Serum n=5</td>
<td>Milk n= 3</td>
<td>Serum</td>
</tr>
<tr>
<td>PFBuS</td>
<td>85% (2%)</td>
<td>79% (4%)</td>
<td>0.7</td>
</tr>
<tr>
<td>PFHxS</td>
<td>89% (3%)</td>
<td>81% (3%)</td>
<td>0.2</td>
</tr>
<tr>
<td>PFOS</td>
<td>82% (5%)</td>
<td>83% (3%)</td>
<td>0.2</td>
</tr>
<tr>
<td>THPFOS</td>
<td>53% (4%)</td>
<td>51% (5%)</td>
<td>1.1</td>
</tr>
<tr>
<td>PFDS</td>
<td>39% (9%)</td>
<td>72% (4%)</td>
<td>0.2</td>
</tr>
<tr>
<td>PFHxA</td>
<td>82% (3%)</td>
<td>80% (2%)</td>
<td>0.7</td>
</tr>
<tr>
<td>PFHpA</td>
<td>82% (2%)</td>
<td>84% (4%)</td>
<td>0.3</td>
</tr>
<tr>
<td>PFOA</td>
<td>89% (2%)</td>
<td>82% (4%)</td>
<td>0.4</td>
</tr>
<tr>
<td>PFNA</td>
<td>95% (2%)</td>
<td>77% (2%)</td>
<td>0.2</td>
</tr>
<tr>
<td>PFDA</td>
<td>56% (5%)</td>
<td>43% (27%)</td>
<td>0.1</td>
</tr>
<tr>
<td>PFUnDA</td>
<td>47% (7%)</td>
<td>38% (3%)</td>
<td>0.2</td>
</tr>
<tr>
<td>PFDaDa</td>
<td>41% (11%)</td>
<td>39% (5%)</td>
<td>0.5</td>
</tr>
<tr>
<td>PFOSA</td>
<td>47% (4%)</td>
<td>34% (0%)</td>
<td>0.1</td>
</tr>
</tbody>
</table>
3. Results and discussion

Results for PFOS, PFHxS, PFOSA, PFNA and PFOA in 26 milk samples and 12 serum sample are given in Appendices 1-5.

A summary of the results of 17 individual milk samples is given in Table 3. PFOS and PFHxS were detected in 16 samples at the mean concentrations 0.172 ng/mL and 0.068 ng/mL, respectively. PFOSA were detected in nine samples with the mean concentration 0.012 ng/mL and PFNA were detected in two samples (0.020 and 0.014 ng/mL). The limits of detection (LOD) were between 0.005 ng/mL and 0.010 ng/mL, except for PFHxA and PFHpA, which were an order of magnitude higher (0.1 ng/mL). Traces in the procedural blanks were seen for several of the compounds (Table 2). Relatively high blank level were obtained for PFOA (0.209 ng/mL) with the consequence that the blank level was higher than 50% of the found concentration for all samples except one.

TABLE 3. Descriptive statistics of 5 PFCs in 17 individual breast milk samples collected from primipara Swedish women, 2003-2005. Concentrations are in ng/mL.

<table>
<thead>
<tr>
<th></th>
<th>PFHxS</th>
<th>PFOS</th>
<th>PFOSA</th>
<th>PFOA</th>
<th>PFNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>N &gt; LOD</td>
<td>16</td>
<td>16</td>
<td>9</td>
<td>1^a</td>
<td>2</td>
</tr>
<tr>
<td>Range</td>
<td>&lt;0.010-0.172</td>
<td>&lt;0.050-0.465</td>
<td>&lt;0.007-0.034</td>
<td>&lt;0.209-0.492</td>
<td>&lt;0.005-0.020</td>
</tr>
<tr>
<td>Mean</td>
<td>0.068</td>
<td>0.172</td>
<td>0.012</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Std dev</td>
<td>0.050</td>
<td>0.112</td>
<td>0.009</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Median</td>
<td>0.058</td>
<td>0.121</td>
<td>0.009</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

^a Additional 12 samples were above blank level but the blank level was more than 50% of the found concentrations.

TABLE 4. Descriptive statistics of 5 PFCs in 12 individual serum samples collected from primipara Swedish women, 2003-2005. Concentrations are in ng/mL.

<table>
<thead>
<tr>
<th></th>
<th>PFHxS</th>
<th>PFOS</th>
<th>PFOSA</th>
<th>PFOA</th>
<th>PFNA</th>
<th>PFDA</th>
<th>PFUnDA</th>
</tr>
</thead>
<tbody>
<tr>
<td>N &gt; LOD</td>
<td>12</td>
<td>12</td>
<td>9</td>
<td>12</td>
<td>12</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>Range</td>
<td>1.78-11.8</td>
<td>8.20-48.0</td>
<td>&lt;0.10-0.49</td>
<td>2.39-5.31</td>
<td>0.43-2.47</td>
<td>0.27-1.78</td>
<td>0.20-1.49</td>
</tr>
<tr>
<td>Mean</td>
<td>4.67</td>
<td>20.7</td>
<td>0.24</td>
<td>3.78</td>
<td>0.80</td>
<td>0.53</td>
<td>0.40</td>
</tr>
<tr>
<td>Std dev</td>
<td>2.85</td>
<td>10.5</td>
<td>0.16</td>
<td>1.0</td>
<td>0.55</td>
<td>0.41</td>
<td>0.35</td>
</tr>
<tr>
<td>Median</td>
<td>4.00</td>
<td>18.7</td>
<td>0.19</td>
<td>3.78</td>
<td>0.63</td>
<td>0.43</td>
<td>0.28</td>
</tr>
</tbody>
</table>
Coupled serum samples were available for 12 of the 17 individual milk samples and a summary of the results can be seen in Table 4. The highest mean concentration were obtained for PFOS (20.7 ng/mL) followed by PFHxS (4.7 ng/mL), PFOA (3.8 ng/mL), PFNA (0.8 ng/mL), PFDA (0.53 ng/mL), PFUnDA (0.40 ng/mL) and PFOSA (0.24 ng/mL). At the current detection limits (0.1-0.5 ng/mL), PFDS was only detected in one sample (0.33 ng/mL). The serum levels are similar to the levels found in previous studies on Swedish human plasma. 

The mean ratio between serum and breast milk (s/bm) concentration was 113 for PFOS, 57 for PFHxS and 23 for PFOSA (Appendices 1-5). Regression analysis of the coupled serum and milk samples shows a strong association (regression coefficient 0.7-0.8) between increasing levels of PFOS and PFHxS in serum and levels in milk (Figure 1). This relationship could not be seen for PFOSA (Figure 2).

FIGURE 1. Regression analysis of PFOS and PFHxS levels in serum and breast milk from primipara women from Sweden, 2004
PFOS and PFHxS were detected in pooled breast milk samples collected each year between 1996 and 2003/2004 from three different regions (Appendices 1-5). The variation between the different years is relatively similar for PFOS and PFHxS (20% and 32% relative standard deviation, respectively). No clear temporal trend can be distinguished although a downward tendency might be reflected in the two last sampling years (Figure 2).

Three women from Örebro collected breast milk at consecutive time points. The interval between donations varied. Times of donation can be seen in Table 5, together with the concentrations of PFOS and PFHxS, which were the only PFCs detected. The levels between sampling days are similar and no trend of decreasing levels with progression of lactation could be seen.

**TABLE 5. Concentrations (ng/mL) of PFOS and PFHxS in breast milk from three women at different time points.**

<table>
<thead>
<tr>
<th>Time of donation</th>
<th>PFOS</th>
<th>PFHxS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Women A (DL0600501)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>First day(^{a})</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>+ 2 days</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>+ 4 days</td>
<td>0.086</td>
<td>nd</td>
</tr>
<tr>
<td>Woman B (DL0600504)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>First day(^{a})</td>
<td>0.060</td>
<td>nd</td>
</tr>
<tr>
<td>+ 59 days</td>
<td>0.060</td>
<td>nd</td>
</tr>
<tr>
<td>Woman C (DL0600513)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>First day(^{a})</td>
<td>0.098</td>
<td>nd</td>
</tr>
<tr>
<td>+ 6 days</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>+ 13 days</td>
<td>0.125</td>
<td>0.015</td>
</tr>
<tr>
<td>+ 20 days</td>
<td>0.104</td>
<td>0.019</td>
</tr>
</tbody>
</table>

\(^{a}\) A = no information, B = 7 weeks after delivery, C = 3 weeks after delivery
Breast milk samples from a total of five regions (Lund, Göteborg, Örebro, Uppsala and Lycksele) were analyzed in this study. Lowest mean concentration of PFHxS and PFOS was found in the Örebro samples, while the highest mean PFHxS concentration was found in the samples from Uppsala and the highest PFOS concentration in the sample from Göteborg. The majority of breast milk samples (18) were from Uppsala with only one pooled sample each from Lund, Göteborg and Lycksele. In addition, the samples from Örebro were collected later after delivery compared to the other samples and a possible variation in concentration with time can not be ruled out. Regional trends are therefore difficult to study.

The selectivity of the method was successfully verified with MS/MS analysis. Qualitative comparison indicated that MS/MS analysis resulted in on average 50% higher concentrations compared to single quadrupole MS. It should be noted that different LC and MS systems were used as well as concentration methods. For the LC-MS analysis, samples were concentrated by evaporation, and in the LC-MS/MS analysis a high volume of the extract was concentrated on-line using a column switch. The differences seen between the methods are not known, but can be multi-factorial.

![PFOS Temporal Trend](image)

![PFHxS Temporal Trend](image)

**FIGURE 3.** Temporal trend of PFOS and PFHxS in breast milk from primipara women in Sweden, 1996-2004.
4. Conclusions

A total of five PFCs were detected in breast milk from Sweden, of which PFOS and PFHxS were found most frequently. In addition, PFOSA, PFNA and PFOA were detected. PFOA was detected in the majority of the samples but the high procedural blank level hampered quantification. The number of compounds (except for PFOSA) and the levels found are in agreement with results from a recently reported study on breast milk from China\textsuperscript{14}. Breast milk levels in this study were 113 times lower compared to serum levels for PFOS, 57 times lower for PFHxS and 23 times lower for PFOSA. There was a strong association between increasing serum concentration and increasing milk concentration for PFOS and PFHxS. During the period 1996-2004, the levels in breast milk were relatively constant with a slightly decreasing trend for the years 2003-2004. No trend was found in levels associated with progression of lactation.

5. Acknowledgment

The study samples were provided by the Swedish National Food Administration and partly from volunteers in the Örebro region, who are all acknowledged. Department of Applied Environmental Research at Stockholm University is kindly acknowledged for letting us use their MS/MS instrument. The study was financial supported by the Swedish Environmental Protection Agency (HÄMI).
References


Appendix 1. Concentrations (ng/mL) of PFOS in serum and breast milk from Sweden together with the serum/breast milk (s/bm) concentration ratio.

### Individual samples

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Appendix 2. Concentrations (ng/mL) of PFHxS in serum and breast milk from Sweden together with the serum/breast milk (s/bm) concentration ratio.

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Appendix 4. Concentrations (ng/mL) of PFNA in serum and breast milk from Sweden together with the serum/breast milk (s/bm) concentration ratio.

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### Pooled samples

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Appendix 5. Concentrations (ng/mL) of PFOA in serum and breast milk from Sweden together with the serum/breast milk (s/bm) concentration ratio.

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